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TITLE: Pre-Clinical Testing of New Hydroxybutyrate Analogues

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT Mitochondria are the powerplants of the cell. They produce the ATP necessary for the neuronsto engage in reactions geared toward their proper function. Mitochondria contain a series of enzymes, in a chain-like array, that pass electrons along this chain via proton motive force which is initiated by complex I, the first of this series of enzymes. Complex I deficiency isconsidered one of the hallmarks of Parkinson's Disease as it contributes greatly to the energy crisis in the neurons. In an earlier study, bypassing this complex I deficiency using D- α -hydroxybutyrate (D α HB) in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of PD, dopamine neurons in the substantia nigra pars compacta were protected. Our goal in this study is to assess the effects of D α HB analogues to ascertain if they are longer-acting compounds than the parent compound. Although obtaining the first and only drug at the moment was quite difficult (it took close to 10 months, we have now initiated our first experiment which is to determine the effective dose to use in future experiments.					
15. SUBJECT TERMS None provided.					
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Introduction

Recently, it has been suggested that Parkinson's Disease (PD) is a multi-faceted disease (1), that is, a disease not based on a single cause, but based in many interacting causes such as genetics (gene alterations), the environment (environmental toxins) and the mitochondria (mitochondrial defects/alterations), as all of these impact the dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). Loss of the DA neurons in the SNpc is considered the main cause, but not the only cause, of the behavioral manifestations of PD (1). One of the most noted and still unanswered questions in PD research is why are some subsets of DA neurons more susceptible to environmental changes than others? Recent evidence suggests that environmental defects, such as those found in mitochondria, contribute to the death of the DA neuron (2). Mitochondria are the powerhouses of the cell and, as such, produce the energy necessary for the cell to function (3). Movement of electrons through the mitochondrial electron transport chain (METC), a series of enzymes, starts with proton motive force at the complex I site, the first of the METC series of enzymes. Electrons move down the chain to eventually produce ATP (4). However, when complex I is compromised, as is reported in PD (5) and demonstrated in the MPTP mouse model of PD (6), mitochondria become dysfunctional as mitochondrial membrane potential collapses, ATP production is reduced and protons no longer travel along the chain. Thus, the neuron experiences energy crisis and respiratory failure, which means that oxidative phosphorylation is compromised and there is an increase in the presence of the superoxide radical (7).

MPTP is the tool of choice for modeling PD as it captures almost all of the hallmarks of PD. MPP⁺, the toxic metabolite of MPTP, accumulates in the mitochondria via the mitochondrial transmembrane potential (8) where it inhibits complex I in the chain. Since inhibition at the complex I site is the beginning of a host of events that are detrimental to the neuron, this area of the mitochondrion offers several possible sites for therapies, i. e. the complex I and II sites.

Several years ago, we evaluated the use of ketone bodies as secondary sources of energy for mitochondria compromised due to blockade at the complex I site of the METC (9). In this early study, using MPTP as the stressor and inhibitor at the complex I site, we found that D- β -hydroxybutyrate (D β HB), a ketone body normally produced by hepatocytes and astrocytes and infused via Alzet pump, protected the substantia nigra pars compacta (SNpc) dopamine (DA) neurons, prevented the development of the motor manifestations of DA neuron loss and enhanced oxidative phosphorylation, all by ramping up the complex II (succinic ubiquinone oxidoreductase dehydrogenase) site (9). Because D β HB is relatively short acting compound, small changes in the molecule

might increase its half-life and negate the use of the Alzet pump. Furthermore, compounds similar to D β HD may be useful in the treatment of PD.

Body of Work

The goal of this study is to fully assess and compare the potency of compounds that are structurally related to D- β -hydroxybutyrate, (β OHB), but are synthesized to be longer acting. Previously, we have found that the infusion of β -OHB to MPTP-treated mice protected SNpc DA neurons from MPTP, not by acting against the MPTP-induced blockade of the complex I site of mitochondria, but by ramping up oxidative phosphorylation via a mechanism that is dependent on complex II. **Thus, in SA I, we intend to assess the effects of the new compounds on mitochondria extracted from naïve mice to gauge their effects on a number of normal individual mitochondrial functions and compare these effects to those elicited by β OHB.** Such functions include ATP production, oxygen consumption, membrane potential and HDAC activity. **SAII is to be done in the MPTP mouse model of PD. We are to compare pump dosing versus one injection per day. This is to gauge whether these compounds can act as a stabilizing force in an environmental upheaval situation like PD.**

Key Accomplishments

Our first key accomplishment for this award, and this is indeed major, is finally receiving one of the two discussed D β HB-like compounds. The compounds to be studied have been quite difficult to obtain and the one that we have just received, Glycerol tris(3-hydroxybutyrate) (G3HB), arrived on April 13, 2011, almost an entire year into this award. Through various contacts and through meetings with Dr. xxxx Hashim, a chemist, who contacted several chemists, we finally obtained the G3HB compound from Dr. Neil Boaz, a green chemist at the Eastman Chemical Company in Kingsport, Kentucky. The received compound has a molecular weight of 350.36, is about 94% pure and is water soluble. We have received a total of 80 grams (see attached). Delays in getting the G3HB to us were unavoidable as this compound had to be synthesized several times to obtain the quantity that we need and re-synthesized several times to increase its purity. We are still in negotiations for the second compound.

Our second key accomplishment for this award is the initiation of the pilot studies. Prior to the start of our pilot study, we had to determine whether the G3HB was indeed soluble in water-based solutions as it is quite viscous. For this pilot study, we have just implanted mice (8 per group and per condition) with 14 days Alzet pumps containing

either 1.6 mmol, 0.8, 0.4 and 0.2 mmol G3HB, 1.6 mmol D β HB, for comparison of the proper dose, or saline, this range of doses being based on doses used in our original study (9). Two days after pump implantation, mice (5 per group) are to receive MPTP free base, 18mg/kg intraperitoneally in 4 doses over 8 hours, each dose 2 hours after the previous dose (as per our original protocol). The remaining 3 mice per group are to receive saline only to ascertain the effects of G3HB on the DA neurons in the SNpc. These mice are to be perfused at seven days after the last injection of MPTP, their brains removed and processed for tyrosine hydroxylase (TH) immunostaining..

Reportable Outcomes

There is nothing yet to report.

Conclusion

None, as we have no data.

References

1. Fahn S and Przedborski S. Parkinsonism. In: Merritt's Neurology, 12th Edition, LP Rowland and TA Pedley, eds. Philadelphia, Waters Kluwer/Lippincott, Williams and Wilkins, 2009, pp 751-769.
2. Greenamyre, JT, Sherer TB, Betarbet, R, Panov, AV. Complex I and Parkinson's Disease. IUBMB Life (2001) 52:135-141.
3. Przedborski S. [Pathogenesis of nigral cell death in Parkinson's disease.](#) Parkinsonism Relat Disord (2005) Suppl 1:S3-7.
4. Jacobson J, Duchen MR, Hothersall J, Clark JB, Heales SJ. [Induction of mitochondrial oxidative stress in astrocytes by nitric oxide precedes disruption of energy metabolism.](#) J Neurochem. (2005) 95: 388-395.
5. Banerjee R, Starkov AA, Beal MF, Thomas B. [Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis.](#) Biochim Biophys Acta (2009) 1792: 651-663.
6. Przedborski S, Kostic V, Jackson-Lewis V, Naini AB, Simonetti S, Fahn S, Carlson E, Epstein CJ, Cadet JL. [Transgenic mice with increased Cu/Zn-superoxide dismutase](#)

[activity are resistant to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity.](#) J Neurosci (1992) 12: 1658-1667.

7. Tieu K, Perier C, Vila M, Caspersen C, Zhang HP, Teismann P, Jackson-Lewis V, Stern DM, Yan SD, Przedborski S. [L-3-hydroxyacyl-CoA dehydrogenase II protects in a model of Parkinson's disease.](#) Ann Neurol. 2004 Jul;56(1):51-60.

8. Singh Y, Bhatnagar R, Sidhu GS, Batra JK, Krishna G. [1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine inhibits proton motive force in energized liver mitochondria.](#) Arch Biochem Biophys (1989) 271: 217-222.

9. Tieu K, Perier C, Caspersen C, Teismann P, Wu DC, Yan SD, Naini A, Vila M, Jackson-Lewis V, Ramasamy R, Przedborski S. D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. J Clin Invest (2003) 112: 892-901.

Figures and Tables

Material Safety Data Sheets for Glycerol tris(3-hydroxybutyrate).

EASTMAN

Eastman Chemical Company
Eastman Research Division
P.O. Box 1972
Kingsport, Tennessee 37662

PURITY PROFILE*

25 March 2011

Product Name: Glycerol tris(3-hydroxybutyrate)

Ship to:

Attn: Dr. Serge Przedborski/Dr. Vernice Jackson-Lewis
Columbia University, MNC
P&S 4-401
630 West 168th Street
New York, NY 10032
Tel: (212)305-8689

Date shipped: 11 April 2011
Containers shipped: 1
Weight shipped: 80 g

Lot Number EX001250-031

Properties

Sample

Method(s)

identity
appearance
wt% assay
GC assay

consistent with structure
pale yellow oil
>98%

NMR

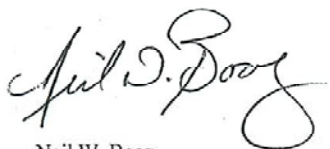
wt% NMR

glycerol tris(3-hydroxybutyrate)
glycerol bis(3-hydroxybutyrate)
residual solvent (ethyl acetate)

93.7%
2.6%
<0.5%

GC derivatization area%
GC derivatization area%
wt% NMR

MW 350.36



Neil W. Boaz
Eastman Representative
423-229-8105
Email nwboaz@eastman.com

**This product is subject to ongoing development. The results provided in this Purity Profile were obtained by analyzing the Batch/Lot described and may or may not be representative of any past or future Batches/Lots. The methodology and/or techniques of analysis used to obtain these results may or may not be validated. The recipient should independently determine whether this product meets their specifications and is technically suitable for their intended purpose. For additional information regarding this product and its analysis, please contact your Eastman representative. This material is NOT for human consumption.*

4/13/11
JC

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MATERIAL SAFETY DATA SHEET

Revision Date: 04/08/2011
MSDSUSA/ANSI/EN/150000072403/Version 2.0

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name	Glyceryl tris(3-hydroxybutyrate)
Product Identification Number(s)	33046-00, E3304601
Manufacturer/Supplier	Eastman Chemical Company 200 South Wilcox Drive Kingsport, TN 37660-5280 US +14232292000
MSDS Prepared by	Eastman Product Safety and Health
Chemical Name	Not applicable
Synonym(s)	985287
Molecular Formula	Not applicable
Molecular Weight	Not applicable
Product Use	research and development sample
OSHA Status	assumed hazardous; not fully investigated

For emergency health, safety, and environmental information, call 1-423-229-4511 or 1-423-229-2000.

For emergency transportation information, in the United States: call CHEMTREC at 800-424-9300 or call 423-229-2000.

2. COMPOSITION INFORMATION ON INGREDIENTS

(Typical composition is given, and it may vary. A certificate of analysis can be provided, if available.)

Weight %	Component	CAS Registry No.
>90%	Glyceryl tris(3-hydroxybutyrate)	135413-30-8
<5%	Glyceryl bis(3-hydroxybutyrate)	Not assigned
<1%	ethyl acetate	141-78-6

3. HAZARDS IDENTIFICATION

WARNING!
THE PHYSICAL-CHEMICAL AND TOXICOLOGICAL PROPERTIES OF THIS MATERIAL HAVE NOT BEEN FULLY INVESTIGATED

HMIS® Hazard Ratings: Health - 2, Flammability - 1, Chemical Reactivity - 0

HMIS® rating involves data interpretations that may vary from company to company. They are intended only for rapid, general identification of the magnitude of the specific hazard. To deal adequately with the safe handling of this material, all the information contained in this MSDS must be considered.

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MATERIAL SAFETY DATA SHEET

Revision Date: 04/08/2011
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exposure limits. If exposure limits have not been established, maintain airborne levels to an acceptable level.

Respiratory Protection: If engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn. Respirator type: Air-purifying respirator with an appropriate, government approved (where applicable), air-purifying filter, cartridge or canister. Contact health and safety professional or manufacturer for specific information.

Eye Protection: Wear safety glasses with side shields (or goggles).

Skin Protection: Wear chemical-resistant gloves, footwear, and protective clothing appropriate for the risk of exposure. Contact health and safety professional or manufacturer for specific information.

Recommended Decontamination Facilities: Eye bath., Washing facilities., Safety Shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical Form: Viscous Liquid

Color: Yellow

Odor: Slight

Specific Gravity: < 1

Boiling Point: 200 °C 0.5 mm Hg

Solubility in Water: Appreciable

Flash Point: > 93 °C (estimated)

Thermal Decomposition Temperature: Thermal stability not tested. Low stability hazard expected at normal operating temperatures.

10. STABILITY AND REACTIVITY

Stability:

Not fully evaluated. Materials containing similar structural groups are normally stable.
Material reacts with Strong oxidizing agents.
Will not occur.

Incompatibility:

Hazardous Polymerization:

11. TOXICOLOGICAL INFORMATION

Acute toxicity data, if available, are listed below. Additional toxicity data may be available on request.

12. ECOLOGICAL INFORMATION

Acute toxicity data, if available, are listed below. Additional toxicity data may be available on request.

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This material has not been tested for environmental effects.

13. DISPOSAL CONSIDERATIONS

Dispose of waste and residues in accordance with local authority requirements. Incinerate. Since emptied containers retain product residue, follow label warnings even after container is emptied.

14. TRANSPORT INFORMATION

Important Note: Shipping descriptions may vary based on mode of transport, quantities, package size, and/or origin and destination. Consult your company's Hazardous Materials/Dangerous Goods expert for information specific to your situation.

DOT (USA)

Class not regulated

Sea - IMDG (International Maritime Dangerous Goods)

Class not regulated

Air - ICAO (International Civil Aviation Organization)

Class not regulated

15. REGULATORY INFORMATION

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This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

WHMIS (Canada) Status: controlled

WHMIS (Canada) Hazard Classification: D/2/B

SARA 311-312 Hazard Classification(s):
immediate (acute) health hazard

SARA 313:

Carcinogenicity Classification (components present at 0.1% or more): none, unless listed below

TSCA (US Toxic Substances Control Act): One or more components of this product are not listed on the TSCA inventory. In the USA, commercial industrial use is restricted to FDA-regulated applications.

16. OTHER INFORMATION

Visit our website at www.EASTMAN.com or email emnmsds@eastman.com

The information contained herein is based on current knowledge and experience; no responsibility is accepted that the information is sufficient or correct in all cases. Users should consider these data only as a supplement to other information. Users should make independent determinations of suitability and completeness of information from all sources to assure proper use and disposal of these materials, the safety and health of employees and customers, and the protection of the environment.

Highlighted areas indicate new or changed information.